Effects of Pulsed UV-Light on Peanut Allergens in Extracts and Liquid Peanut Butter

S.-Y. CHUNG, W. YANG, AND K. KRISHNAMURTHY

ABSTRACT: Pulsed ultraviolet (PUV) light, a nonthermal technology, was used to treat both the peanut extracts and liquid peanut butter. The objective was to determine if such treatment would lead to a reduction in the allergenic properties of the peanut extract and butter. Peanut samples were PUV treated using a Xenon RS-3000C under the following conditions: 3 pulses/s, 14.6 cm from the central axis of the lamp, 4 min (extract) or 3 min (liquid peanut butter). After the treatment, the peanut samples were centrifuged and the supernatants analyzed by SDS-PAGE and competitive inhibition enzyme-linked immunosorbent assay (ciELISA). For comparison, boiling treatments were also performed. SDS-PAGE showed that while boiling treatment had little effect on the peanut allergens, PUV-light-treated samples displayed a reduced solubility or level of peanut allergens (63 kDa). Solubility of another allergen (18 to 20 kDa) was unaffected. Insoluble aggregates formed were responsible for the reduced level of allergens in PUV-light-treated samples. ciELISA showed that untreated samples exhibited an IgE binding 7-fold higher than the PUV-treated samples. It was concluded that PUV light was effective in reducing IgE binding of peanut extracts and liquid peanut butter. The current study provides an approach to the development of a possibly less allergenic peanut product. However, the reduction in actual allergenicity needs to be confirmed by clinical studies.

Keywords: IgE antibodies, peanut allergens, peanut butter, pulsed UV light

Introduction

Pulsed ultraviolet (PUV) light is a nonthermal, high-peak power technology that consists of intense flashes of broad-spectrum white light with wavelengths from 200 nm in the ultraviolet (UV) to 1000 nm in the near-infrared region (Rowan and others 1999). Each pulse may have up to 90000 times the intensity of sunlight at sea level, and may last only a few hundred millionths of a second, and thus a PUV light system can produce very high peak power pulsed light in a very short time. Because of its high peak power, PUV light has been successfully used as a sterilization tool to kill bacteria and fungi in foods and fruits (Krishnamurthy and others 2004, 2007; Lagunas-Solar and others 2006; Bialka and others 2008). The killing effect is 4 to 6 times higher than that of the conventional continuous UV light at the same energy level (Dunn and others 1995; MacGregor and others 1998). The advantage of using PUV light is that PUV light can enhance the shelf life and quality of foods without causing sensory changes (Dunn and others 1995; Gómez-López and others 2005; Lagunas-Solar and others 2006).

While PUV light is generally aimed at killing foodborne organisms, little has been done or is known about its effect on proteins that cause peanut allergy. To date, at least 8 peanut allergens have been identified, of which two, namely, Ara h 1 (63 kDa) and Ara h 2 (18 to 20 kDa) are considered to be the major peanut allergens, because Ara h 1 and Ara h 2 are recognized by 70% to 90% of sensitized individuals (Burks and others 1998). These allergens are reported to react with carbohydrates to form advanced glycation end products (AGE) during heating or roasting of the peanuts, and as a result, the allergenic properties of the peanuts increase (Chung and Champagne 1999, 2001; Maleki and others 2000). To reduce the allergenic properties of peanut allergens, several approaches such as treatments with peroxidase, polypheol oxidase/caffeic acid, copper/hydrogen peroxide, and phytic acid have been developed in our laboratory (Chung and others 2004, 2005, 2006; Chung and Champagne 2007).

In this study, we investigated the feasibility of using PUV light to lower the allergenic properties of peanut extracts and liquid peanut butter. The rationale was that as in other high peak power technologies such as pulsed electric field and matrix-assisted laser pulsed evaporation (Castro and others 2001; Jelinek and others 2007; Wei and others 2007), PUV light may cause protein insolubility, and thus may lead to a reduction in the levels of peanut allergens in the extracts and liquid peanut butter. Therefore, the objective of this study was to treat the peanut extracts and liquid peanut butter with PUV light and determine if the immunoglobulin E (IgE) binding was reduced after treatment. For comparison, boiling treatment was also performed. In this study, whole peanut seeds were not used and treated because PUV light is incapable of penetrating the seed coat to reach and affect the inner peanut proteins.

Materials and Methods

Materials

Tris buffer saline (TBS), 96-well microtiter plates, ō-phenylenediamine, and Tween 20 were purchased from Sigma Co. (St. Louis, Mo., U.S.A.). Tris-glycine precast gels (4% to 20%), gel electrophoresis apparatus, reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and goat antihuman immunoglobulin E (IgE)-peroxidase were purchased from Invitrogen (Carlsbad, Calif., U.S.A.). Superblock blocking buffer, GelCode Blue Stain Reagent, and bicinchoninic acid (BCA)-protein assay kit were purchased from Pierce Chemical Co. (Rockford, Ill., U.S.A.). Human plasmas from 3 individuals

MS 20070938 Submitted 12/17/2007, Accepted 3/26/2008. *Author Chung is with U.S. Dept. of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124, U.S.A. Authors Yang and Krishnamurthy are with Dept. of Food & Animal Science., P.O. Box 1628, Alabama A&M Univ., Normal, AL 35762, U.S.A. Direct inquiries to author Chung (E-mail: siyin.chung@ars.usda.gov).*

doi: 10.1111/j.1750-3841.2008.00784.x

with peanut allergy (determined by CAP-FEIA assay for IgE) were obtained from Plasma La Intl. (Everett, Wash., U.S.A.). Raw peanuts and natural peanut butter (creamy and nonhydrogenated) were purchased from a local food store. A Xenon RS-3000C (PUV light equipment) was purchased from Xenon Corp. (Woburn, Mass., U.S.A.) and located at Alabama A&M Univ., Normal, Ala., U.S.A.

Preparation of peanut extracts and liquid peanut butter

Peanut extracts in 20 mM Tris buffer, pH 7 were prepared from defatted raw peanut meals as previously described (Chung and Champagne 2007), and then subjected to PUV light and boiling treatment as described in the following section. Liquid peanut butter was prepared by stirring natural peanut butter (nonhydrogenated; oil partially removed) in 20 mM Tris buffer, pH 7 at a ratio (1:2) (w:v) at room temperature for 60 min (Chung and Champagne 2007). The peanut butter slurries were then subjected to PUV light as described subsequently.

PUV light and boiling treatment of peanut extracts

A Xenon Steripulse XL-3000® (Figure 1) was used for carrying out the pulsed UV-light treatments. This system produced a broadband spectrum of 200 to 1100 nm with approximately 54%, 26%, and 20% energy in the ultraviolet, visible, and infrared regions, respectively. Three pulses per second with a width of 360 μs was produced. As per the manufacturer's specification, the system produced an energy level of 1.27 J/cm^2 at 7.6 cm below the central axis of the pulsed UV lamp. The system essentially consists of a pulsed UV lamp, a cooling blower, a treatment chamber, and the control module. The distance between the sample and the PUV lamp can be changed using adjustable trays. Preliminary studies indicated that PUV light was effective under the following conditions: 4 min, and 14.6 cm from the central axis of the lamp. As per previous studies, the total available energy at 14.6 cm was determined to be 0.92 J/cm^2/s (Krishnamurthy 2006). Therefore, a 4-min treatment would provide the maximum energy level of 220.8 J/cm^2. However, the actual portion of the energy absorbed by the sample is unknown. Under this condition, peanut extracts (5 mg/mL, 10 mL each in an aluminum dish with a diameter of 7 cm) were treated with and without PUV light (samples were stirred immediately before and after treatments), using the above-mentioned PUV system. For boiling treatment, vials containing the peanut extracts (5 mg/mL, 500 μL each) were placed in a boiling water bath for 4 min. After treatment, the extracts were centrifuged at 8000 × g for 10 min, and the supernatants were subjected to SDS-PAGE and ciELISA as described subsequently. Protein concentration was determined using the BCA kit assay.

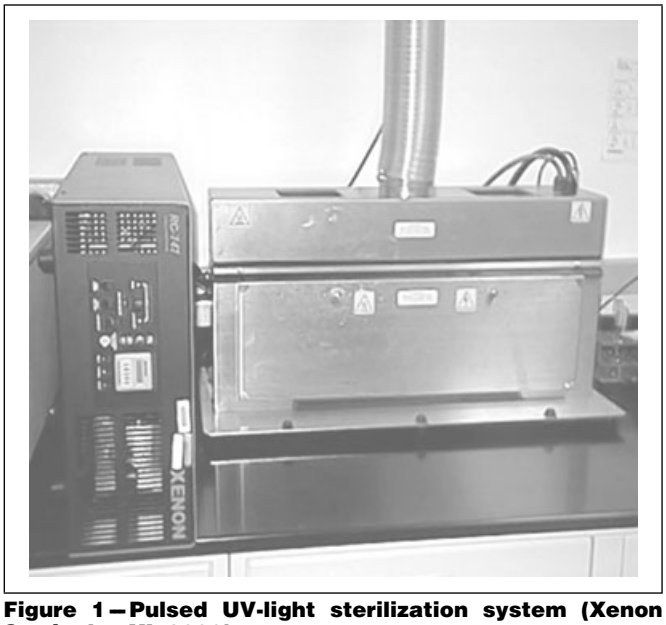

Figure 1—Pulsed UV-light sterilization system (Xenon Steripulse XL-3000).

Treatment of liquid peanut butter with PUV light

Liquid peanut butter samples were treated with PUV light under the same condition as described previously, except that the treatment time was changed to 3 min to prevent off-flavor formation. The maximum energy available was estimated to be 165.6 J/cm^2 as per earlier discussion (Krishnamurthy 2006). After the treatment, the liquid samples were centrifuged at 8000 × g for 10 min, and the supernatants were subjected to SDS-PAGE and ciELISA as described in the following sections.

SDS-PAGE of treated peanut extracts and liquid peanut butter

Supernatants from the above PUV- and boiling-treated extracts and liquid peanut butter were applied to SDS-PAGE under a nonreducing condition. SDS-PAGE was performed as previously described (Chung and Champagne 2007), using Tris-glycine precast gels (4% to 20%) and a Novex gel electrophoresis apparatus. Gels were stained with GelCode Blue, and destained with water.

Determination of IgE binding of treated peanut extracts and liquid peanut butter

A competitive inhibition enzyme-linked immunosorbent assay (ciELISA) was carried out ($n = 3$) as previously described (Chung and Champagne 2007). Briefly, 50 μL of a peanut sample (treated or untreated) at 0.01 to 10 μg/mL were mixed with a pooled human plasma (1:20, 50 μL) obtained from 3 patients with clinical allergy to peanuts, and then incubated for 30 min in a plate coated with a raw peanut extract. The immunoglobulin E (IgE) antibodies, which were attached to the plate, were then detected using a goat antihuman IgE peroxidase conjugate (1:1000, 100 μL) and a substrate solution (100 μL) containing *o*-phenylenediamine (0.5 mg/mL) and 0.03% hydrogen peroxide in 0.1 M citrate buffer, pH 5.5. After stopping the enzyme reaction with 4 N sulfuric acid (50 μL), the absorbance was read at 490 nm with a CERES 900C plate reader (Bio-Tek Instruments Inc., Winooski, Vt., U.S.A.). All samples except the substrate were diluted in Superblock:TBS/Tween 20 (1:1). The absorbance value of a sample containing IgE antibodies and the peanut sample was represented by B, while Bo represented the absorbance value of a control containing IgE only. Values are means of triplicate measurements ($n = 3$). Statistical analyses were performed using a Student's t-test at a $P < 0.05$ level of significance.

Results and Discussions

SDS-PAGE of PUV-treated peanut extracts and liquid peanut butter

After PUV light treatment, both peanut extracts and liquid peanut butter exhibited a change in the volume. The volume was reduced by approximately 40% as a result of water evaporation. The PUV-treated samples were centrifuged or allowed to stand for the solids to settle, and the supernatants were then subjected to SDS-PAGE.

A typical SDS-PAGE profile of the PUV-treated peanut extract is shown in Figure 2A. Protein bands corresponding to 63 kDa allergen and 50 kDa protein were hardly seen in the PUV-treated extract, as compared to the control, while bands corresponding to 18- to 20-kDa allergen remained clearly visible. This suggests that only the 63 kDa allergen was affected by PUV light. Its disappearance in SDS-PAGE indicated that the majority of 63 kDa allergens were not in the PUV-treated peanut extract as soluble proteins, but rather they existed as insoluble aggregates or precipitates. This phenomenon of aggregation has been demonstrated by several studies that utilize similar high-peak-power technologies such as pulsed electric field and matrix-assisted pulsed laser evaporation to inactivate enzymes (Castro and others 2001; Jelinek and others 2007; Wei and others 2007). In those studies, the researchers demonstrated that aggregation was a result of protein denaturation. Whether PUV light in this study caused protein denaturation is not known.

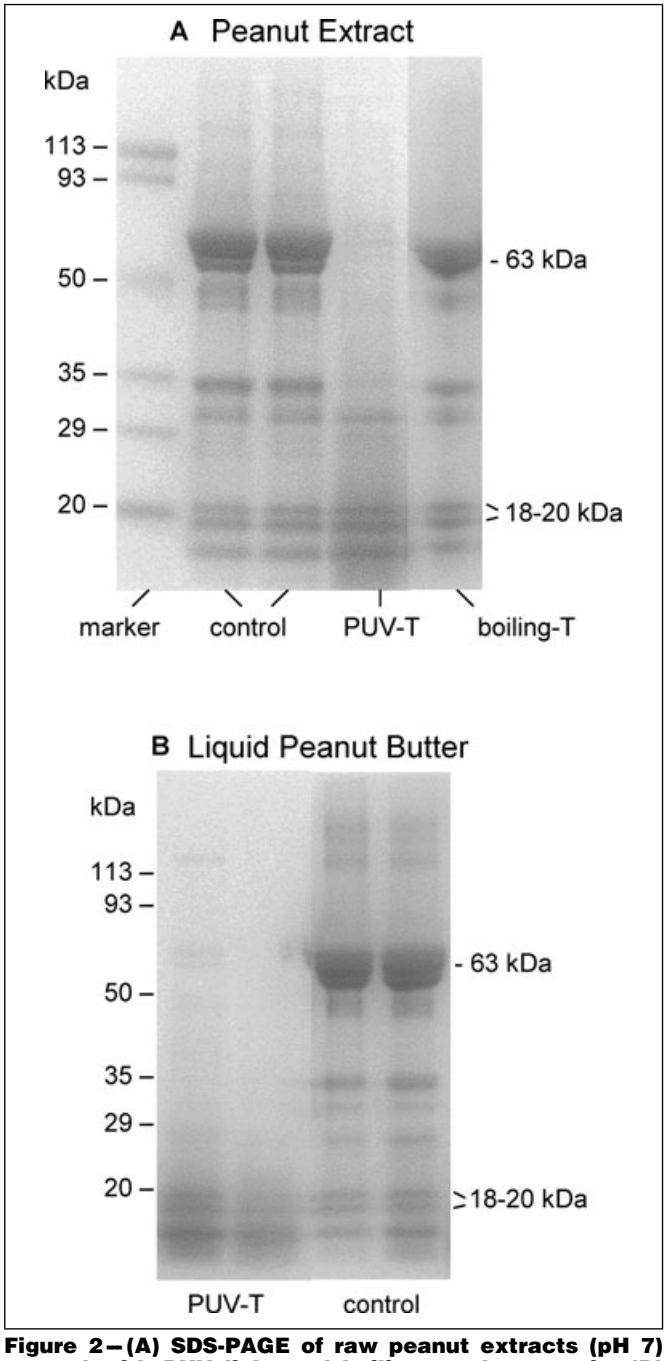

Figure 2—(A) SDS-PAGE of raw peanut extracts (pH 7) treated with PUV light and boiling, each at 4 min. (B) SDS-PAGE of PUV-treated liquid peanut butter. After the treatment, the peanut samples were centrifuged, and the supernatants were subjected to SDS-PAGE. T = treated.

The above-mentioned finding indicated that the 18- to 20-kDa allergen does not form aggregates because, as shown in Figure 2A, the majority of 18- to 20-kDa allergens still remained soluble despite PUV light treatment. Cases where these allergens are simultaneously affected have been reported (Chung and others 2004, 2005; Chung and Champagne 2007). In those studies, the allergens were not denatured but rather cross-linked with each other or formed insoluble complexes with natural chemical compounds, the end result of which led to a reduction in the levels of the allergens and, eventually, the IgE binding of the treated peanut extracts. In summary, this study showed a reduced level of soluble peanut allergens in the PUV-treated peanut extracts. The IgE binding of these extracts probably would be reduced as well (see IgE binding subsequently).

Because proteins can also aggregate when treated with boiling (Mills and others 2001), we wanted to determine if the boiling treatment affects peanut allergens in the same way as PUV light (treatment time was the same in both cases). Beyer and others (2001) also kept the treatment time the same for the cooking methods (boiling, roasting, and frying) used for treatment of peanuts. A longer treatment time was not performed in this study because it created off-flavors problems, especially with the liquid peanut butter (an observation). Figure 2A shows the typical SDS-PAGE profile of the boiling-treated peanut extract compared to that of the PUV-treated. Despite the formation of aggregates, the boiling-treated extract displayed no missing bands (that is, 63 and 50 kDa) that occurred earlier to the PUV-treated extract. Instead, it was similar to the control profile. This suggests that despite the boiling treatment, the majority of the allergens were still soluble and did not exist as aggregates. In this case, it appears that PUV light was much more efficient than boiling in the aggregation of the 63-kDa allergen. The formation of aggregates is important because they can eventually be removed to produce a peanut extract with a lower level of peanut allergens and, therefore, a lower allergenic property.

Figure 2B presents a typical SDS-PAGE profile of the soluble fraction of the PUV-treated liquid peanut butter. The result also showed the disappearance of the 63-kDa allergen and 50-kDa protein compared to the control. Other proteins, except 18- to 20-kDa allergen, were also missing. The data suggest that the solubility or level of 63-kDa allergens in the liquid peanut butter was reduced after PUV light treatment. This reduction in peanut allergens was a result of aggregation of the peanut allergens, as indicated previously. These aggregates were easily seen when the supernatant from PUV-treated liquid peanut butter was allowed to stand at 4 °C. These should not be confused with the cryoproteins, which are known to aggregate in the cold (at 4 °C) and are reversibly soluble when incubated at room temperature. The aggregates from PUV light treatment are different from cryoproteins because the former are irreversibly insoluble at room temperature. The test results indicated that the aggregates were insoluble in buffers, 1 M NaCl, or 2 M urea (data not shown). Digestion by enzymes such as pepsin or trypsin was also unsuccessful due to solubility problems. SDS-PAGE of the aggregates showed that the aggregates contained mostly the 63-kDa allergen and 50-kDa protein and very little 18- to 20-kDa allergen (Figure 3). This matched the profile of the supernatant (applied

to SDS-PAGE after removal of the aggregates), which was shown to contain very little 63/50 kDa but 18- to 20-kDa bands (Figure 3). In this case, the control was a mirror of both aggregates and supernatant. In summary, the result indicated that the liquid peanut butter exhibited a reduced solubility or level of peanut allergens after PUV treatment.

IgE binding of PUV-treated extracts and liquid peanut butter

The above-mentioned finding of the PUV-treated peanut extracts and liquid peanut butter with a reduced level of peanut allergens led us to postulate that the IgE binding of the PUV-treated peanut samples may be reduced. To test the postulation, a competitive inhibition ELISA (ciELISA) was performed using a pooled human plasma from 3 patients with clinical allergy to peanuts containing IgE antibodies against peanut allergens. In the assay, treated and untreated peanut extracts and liquid peanut butter were each tested for their inhibitory effects on the IgE antibodies. In this case, the higher the inhibitory effect, the higher the IgE binding capacity of the peanut sample. The results (Figure 4A) showed that the inhibitory effect was less pronounced with the PUV-treated peanut extract than with the control. When the boiling-treated extract was tested, its inhibitory effect on IgE binding was not much different from that of the control, but instead was higher than that of the PUV-treated extract (Figure 4A). In the case of liquid peanut butter, the inhibitory effect was also less pronounced with the PUV-treated liquid peanut butter (Figure 4B). All this suggests that the IgE binding ability of the PUV-treated extract or liquid peanut butter, except the boiling-treated, was reduced as compared to the control. The result is in agreement with the above-mentioned SDS-PAGE data (Figure 2A and 2B) and in support of the postulation that a lower IgE binding is a result of the reduced level of peanut allergens in the treated extract.

The values of IC_{50}, defined as the concentration of proteins (inhibitors) required to inhibit IgE binding by 50%, for the PUV-treated extract and the control (or boiling-T) were 4 and 0.7 μg/mL, respectively (Figure 4A). Similar values (4 and 0.6 μg/mL) were obtained, respectively, with the PUV-treated liquid peanut butter and the control. The significant difference in IC_{50} between the

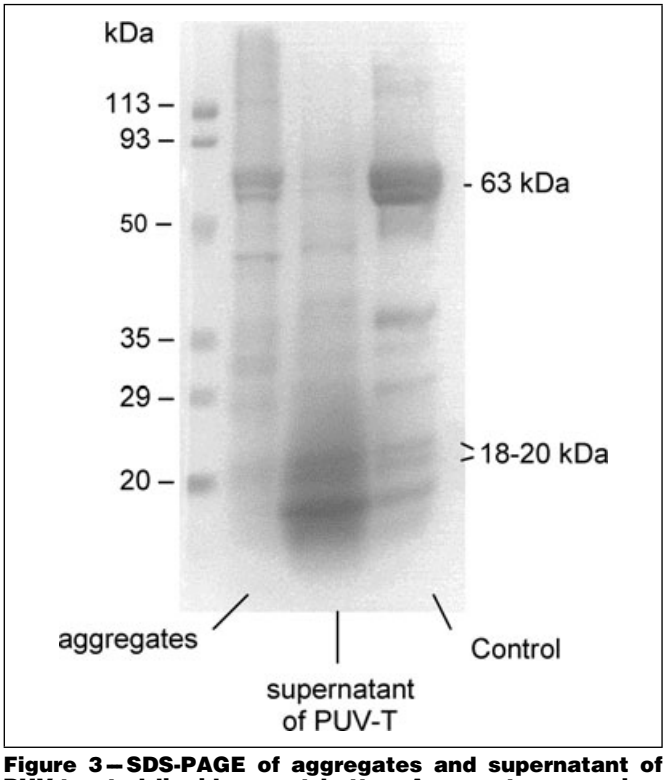

Figure 3—SDS-PAGE of aggregates and supernatant of PUV-treated liquid peanut butter. Aggregates were isolated from the supernatant of the PUV-treated liquid peanut butter sample after centrifugation. The supernatant was applied to SDS-PAGE after the removal of the aggregates. Both were compared to the untreated or control. T = treated.

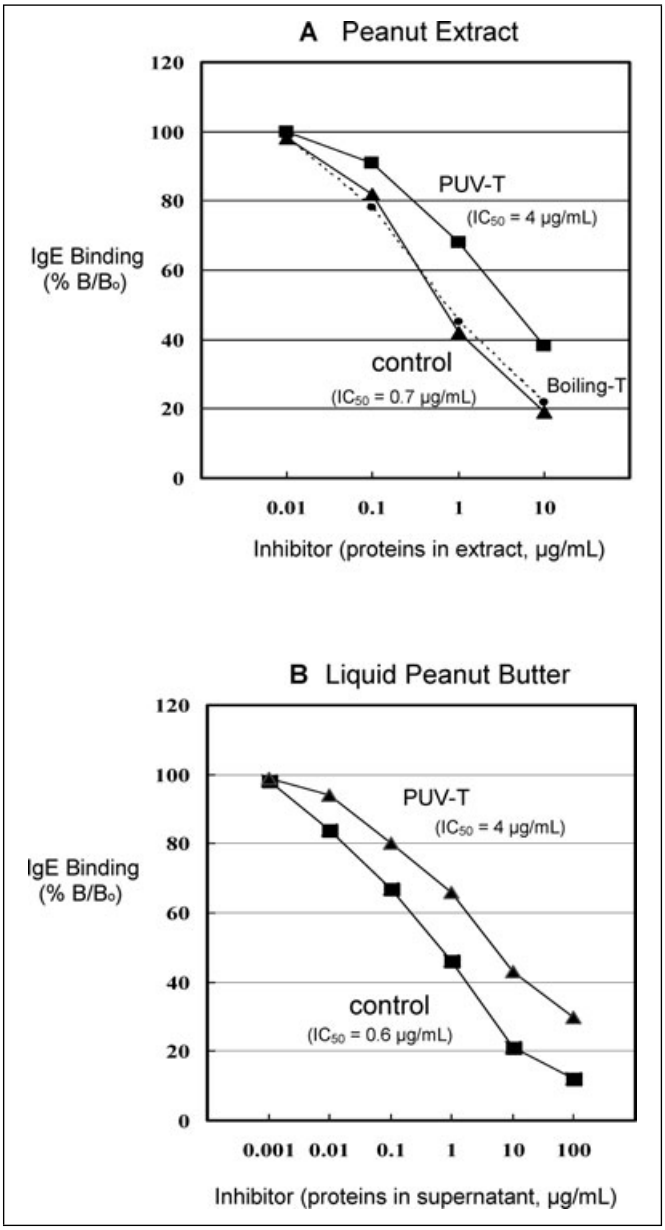

Figure 4—(A) IgE binding of PUV- and boiling-treated and untreated peanut extracts. (B) IgE binding of PUV-treated and untreated liquid peanut butter. IgE binding was performed in a ciELISA using a pooled human plasma (1:20) from peanut-allergic individuals. IgE was detected using a goat antihuman IgE peroxidase conjugate (1:1000) and a substrate solution containing *o*-phenylenediamine (0.5 mg/mL) and 0.03% hydrogen peroxide, pH 5.5. IC_{50} is defined as the concentration of proteins (inhibitors) required to inhibit IgE binding by 50%. Values at 0.01 to 100 μg/mL are means of triplicate measurements ($n = 3$). Values of PUV-T at 0.1 to 100 μg/mL are significantly different from those of the control ($P < 0.05$).

PUV-treated and the control ($P < 0.05$) indicated that the IgE binding of the control was approximately 6- to 7-fold higher than that of the PUV-treated sample. This reduced IgE binding of the PUV-treated may in fact affect the clinical reactivity.

Potential applications

New regular (that is, nonhypoallergenic) peanut product such as liquid peanut butter is currently being marketed and sold by Superior Nut Co. (Cambridge, Mass., U.S.A.) as a topping or flavor in smoothies and shakes. However, such a regular peanut product may create a problem for children or people who are at risk of peanut allergy. This study provides an alternative or approach to the development of a possibly less allergenic peanut product.

Conclusions

PUV-light treatment resulted in reduced solubility of peanut allergens (63 kDa) in the peanut extracts and liquid peanut butter. The solubility of another peanut allergen (18 to 20 kDa) was not affected under the conditions tested. The effect of PUV light on the peanut allergen was demonstrated by SDS-PAGE where bands corresponding to 63 kDa were missing in the PUV-treated samples. Aggregates that formed in the treated samples accounted for the peanut allergens missing. The aggregates were irreversibly insoluble in buffers 1 M NaCl or 2 M urea. Boiling treatment also led to aggregate formation, but the majority of 63-kDa allergens did not exist as aggregates. This indicates that unlike PUV light, boiling treatment had little effect on the peanut allergen. This was further demonstrated in ciELISA where the boiling-treated extract, like the control, exhibited a higher IgE binding than the PUV-treated extract. ciELISA of the PUV-treated liquid peanut butter indicated that the IgE binding of the control was approximately 7-fold higher than that of the PUV-treated liquid peanut butter. The current study thus provides an approach to the development of a peanut product with a possibly lower allergenic property. However, the reduction in actual allergenicity needs to be confirmed by clinical studies.

Acknowledgment

The authors thank Shawndrika Reed and Elena Batista for their technical support.

References

Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks W, Sampson HA. 2001. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 107(6):1077–81.
Bialka KL, Demirci A, Puri VM. 2008. Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. J Food Eng 85(3):444–9.
Burks AW, Sampson HA, Bannon GA. 1998. Peanut allergens: review article series II. Allergy 53:725–30.
Castro AJ, Swanson BG, Barbosa-Canovas GV, Dunker AK. 2001. Pulsed electric field denaturation of bovine alkaline phosphatase. In: Barbosa-Canovas GV, Zhang QH, editors. Pulsed electric fields in food processing. Lancaster, Pa.: Technomic Publishing. p 83–103.
Chung SY, Champagne ET. 1999. Allergenicity of Maillard reaction products from peanut proteins. J Agric Food Chem 47:5227–31.
Chung SY, Champagne ET. 2001. Association of end-product adducts with increased IgE binding of roasted peanuts. J Agric Food Chem 49:3911–6.
Chung SY, Champagne ET. 2007. Effects of phytic acid on peanut allergens and allergenic properties of extracts. J Agric Food Chem 55:9054–8.
Chung SY, Maleki SJ, Champagne ET. 2004. Allergenic properties of roasted peanut allergens may be reduced by peroxidase. J Agric Food Chem 52:4541–5.
Chung SY, Kato Y, Champagne ET. 2005. Polyphenol oxidase/caffeic acid may reduce the allergenic properties of peanut allergens. J Sci Food Agric 85:2631–7.
Chung SY, Kato Y, Champagne ET. 2006. Reducing the allergenic properties of peanut allergens by copper/hydrogen peroxide. In: Singh VK, Govil JN, Ahmad K, Sharma RK, editors. Recent progress in medicinal plants. Vol. 15: natural products. Houston, Tex.: Studium Press LLC. p 443–53.
Dunn J, Ott T, Clark W. 1995. Pulsed-light treatment of food and packaging. Food Technol 49(9):95–8.
Gómez-López VM, Devlieghere F, Bonduelle V, Debevere J. 2005. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. Int J Food Microbiol 103(1):79–89.
Jelinek M, Remsa J, Brynda E, Houska M, Kocourek T. 2007. Thin layers of bovine serum by matrix assisted pulsed laser evaporation. Appl Sur Sci 254(4):1240–3.
Krishnamurthy K. 2006. Decontamination of milk and water by pulsed UV light and infrared heating [PhD diss.]. Dept. of Agricultural and Biological Engineering, Pennsylvania State Univ., University Park, Pa.
Krishnamurthy K, Demirci A, Irudayaraj J. 2004. Inactivation of *Staphylococcus aureus* by pulsed UV-light sterilization. J Food Prot 67(5):1027–30.
Krishnamurthy K, Demirci A, Irudayaraj JM. 2007. Inactivation of *Staphylococcus aureus* in milk using pulsed UV-light treatment system. J Food Sci 72(7):233–9.
Lagunas-Solar MC, Pina C, MacDonald JD, Bolkan L. 2006. Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. J Food Prot 69(2):376–84.
MacGregor SJ, Rowan NJ, Macllvaney L, Anderson JG, Fouracre RA, Farish O. 1998. Light inactivation of food-related pathogenic bacteria using a pulsed power source. Lett Appl Microbiol 27:67–70.
Maleki SJ, Chung SY, Champagne ET, Raufman JP. 2000. The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immunol 106:763–8.
Mills ENC, Huang L, Noel TR, Gunning AP, Morris VJ. 2001. Formation of thermally induced aggregates of the soya globulin β-conglycinin. Biochem Biophys Acta—Protein Struct Mol Enzymol 1547(2):339–50.
Rowan NJ, MacGregor SJ, Anderson JG, Fouracre RA, Macllvaney L, Farish O. 1999. Pulsed-light inactivation of food-related microorganism. Appl Environ Microbiol 65(3):1312–5.
Wei ZW, Ruijin YR, Rongrong LR, Yali TY, Wenbin ZW. 2007. Investigation of the mechanisms of pulsed electric fields on inactivation of enzyme: lysozyme. J Agric Food Chem 55:9850–8.